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REPORT ON

DEVELOPMENT OF A PRACTICABLE SYSTEM FOR MONITORING THE MICROBIOLOGICAL CHARACTERISTICS OF FOREST LAKES

U.S. FOREST SERVICE 16-444-CA

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EXPERIMENT STATION

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Chapter 1

INTRODUCTION

Authorization

This study was sponsored by the Eisenhower Consortium for Western Environmental Forestry Research. The project was supported by the United States Department of Agriculture, Rocky Mountain Forest and Range Experiment Station under a cooperative agreement, 16-444-CA, with Arizona State University.

Need for Study

Increased recreational use of National Forest Lakes and the consequent increased demand on recreational facilities may adversely effect the aquatic environment. Expansion of recreational facilities is needed at these lakes to accommodate the demand, but it is not known what environmental changes may occur from the development and increased use of recreational facilities.

The development of a forest lake environment for recreational activities may result in adverse environmental changes in the lake. Several examples of what may occur:

1) The clearing of trees for roads and campsites could possibly cause a slight increase of runoff into the lakes and a resultant increase of the total solids and turbidity in the lake. Runoff may also carry with it nutrients and organic residues from campsites and other activities. The effects of runoff are subtle and may result in adverse chemical and biological events in the lake.
2) Solid wastes such as waste paper, containers, and food scraps may find their way into the lake from various activities. Besides causing aesthetic deterioration, these substances may contribute to an oxygen demand or other adversities if concentrated in certain areas.
3) Runoff and leakage from pit privies can occur if their operation is not closely scrutinized.

These effects may be innocuous and easily assimilated by the lake, or they may be deleterious and result in adverse environmental changes. What actually is occurring may be ascertained to enable proper action by all persons involved.

The specific biological change in water quality occurring will be monitored by comparing a lightly used, undeveloped lake (Bear Canyon Lake) with a heavily used, developed one (Woods Canyon Lake).

The two lakes chosen for this study are Bear Canyon Lake and Woods Canyon Lake, both in the Sitgreaves National Forest. Bear Canyon Lake is relatively undeveloped, has no recreational facilities, and is reached via 12 miles of unpaved road. Conversely, Woods Canyon Lake is developed, having 78 designated campsites near the lake and another 70 campsites within 1 mile from the lake. Boat launching facilities are also available at Woods Canyon Lake, which is easily accessible to the public by paved roads. Bear Canyon Lake will serve as the control, representing a relatively pristine lake with minimal effects from recreational activities. Woods Canyon Lake is thus the experimental unknown, and any deviations of selected water quality parameters can be attributed to development of recreational activities.

The lakes are located in Coconino County, Arizona approximately 44 miles Northeast of Payson on the North slope of the Mogollon Rim. The area is subject to heavy Summer rains and Winter snows; precipitation averages about 20 inches per year. Air temperatures range from 100°F in Summer to -30°F in Winter. Most of the runoff entering these lakes comes from Winter snows melting in late Spring, although the late Summer rains may also contribute significant quantities of water. The vegetative cover in this region is Ponderosa pine.

Both lakes are man made. Woods Canyon Lake was completed in 1956 and Bear Canyon Lake in 1964. The maximum depth of Woods Canyon Lake is 36 feet and Bear Canyon Lake has a maximum depth of 25 feet.

Objectives

The overall objective of this study is to determine what biological changes in the lake water quality may occur as a result of increased recreational use, and whether these changes are innocuous or deleterious. From this information, guidelines governing development of the recreational facilities may be established to keep the activities at a level which will not alter the lake's environment significantly from its natural condition.

Specific objectives of this study are:

1. To determine the seasonal effects of recreation activities on the bacteriological quality of a forest lake.

2. To ascertain what types of development and recreational activities contribute to biological pollution.

3. To develop a system of routine analysis which can be performed to monitor changes in water quality.

Methods

The specific methods are as follows:

1. To review the history of these lakes by inspecting previous reports, interviewing selected personnel, etc.

2. To observe the populations that are utilizing the recreactional facilities at these lakes at selected time intervals. This will be accomplished by periodically checking the number of campsites at each lake. The Forest Service also keeps records of recreational use at these lakes and this information will be solicited.

3. Samples from both lakes will be collected on a periodic basis from several areas of each including near shore bottom samples commencing in early May and continuing through October. Bacterial viable counts will be made of all samples utilizing a simple peptone medium known to select for normally occurring water bacteria. Dilution samples will also be incubated and innumerated anaerobically using portable gas-pac equipment to provide an aerobic/ anaerobic ratio indicative of potential increasing anaerobic conditions. Isolations of bacterial types will be made from the above plates and these isolates identified as to genera and biotypes. Enrichment media to select for sulfur, iron, nitrifying and photosynthetic bacteria will be inoculated with appropriate samples to provide an indication of biological activities in the two lakes.

By analysis of the bacterial counts obtained from shore and mid-lake samples under variable oxygen and temperature condition a comparison of the two lakes as they are effected by seasonal changes will be obtained. A

correlation of the use activity at the two lakes with the bacterial variation using the above parameters combined with routine estimates of coliform and fecal streptococci will provide an indication of the effect of development and recreational activities on the lake.

The information obtained concerning the physical and biological limits of tolerance (temperature, pH, salt, etc.) of isolated bacteria from both lakes will provide a rationale for developing a monitoring system, through use of selective conditions, that can be used to ascertain changes in water quality.

The above tests are indicative of the quality of the lake water. These tests are not limited to exemplifying adversities, but will also be indicative of high quality water.

Previous studies existed only for Woods Canyon Lake (Kemmerer, 1967) and these were concerned only with physical data and the algae productivity of the lake. No data on bacterial flora was available for these lakes prior to this study. A correllary study on physical parameters of the two lakes was done over the same period and using similar sampling sites by Dr. Burton Segall as a separate project. The characteristics of the two lakes are compared in Table 1.

Table 1

Morphometric Features of Woods Canyon and Bear Canyon Lakes

	Woods Canyon Lake	Bear Canyon Lake
Location	Mogollon Rim	Mogollon Rim
Elevation	7500 ft	7200 ft
Origin	Man made	Man made
Age	18 years	10 years
Surface area	51 acres	65 acres
Volume	1013 acre-feet	1430 acre-feet
Mean depth (V/A)	19.8 feet	22.0 feet
Drainage area	8.8 square miles	2.0 square miles
Purpose	Recreation	Recreation
Volume of Epilimnion,E	493 acre-feet	649 acre-feet
Volume of Hypolimnion,H	79 acre-feet	371 acre-feet
E/H	6.2	1.8

Chapter 2

MATERIALS AND METHODS

Collection of Samples:

Samples were collected in sterile 8 ounce plastic bottles by immersing the uncapped bottle a few centimeters below the surface. Shore samples were taken one to two feet from the shore using rocks or stumps for support to avoid the bottom mud. Mid-lake samples were collected from a rubber raft. Mud samples were collected from muds near shore in sterile bottles. All samples were cooled as soon as possible, usually less than one hour, dependent on collecting conditions. All samples were cultured for isolation in less than six hours following their collection.

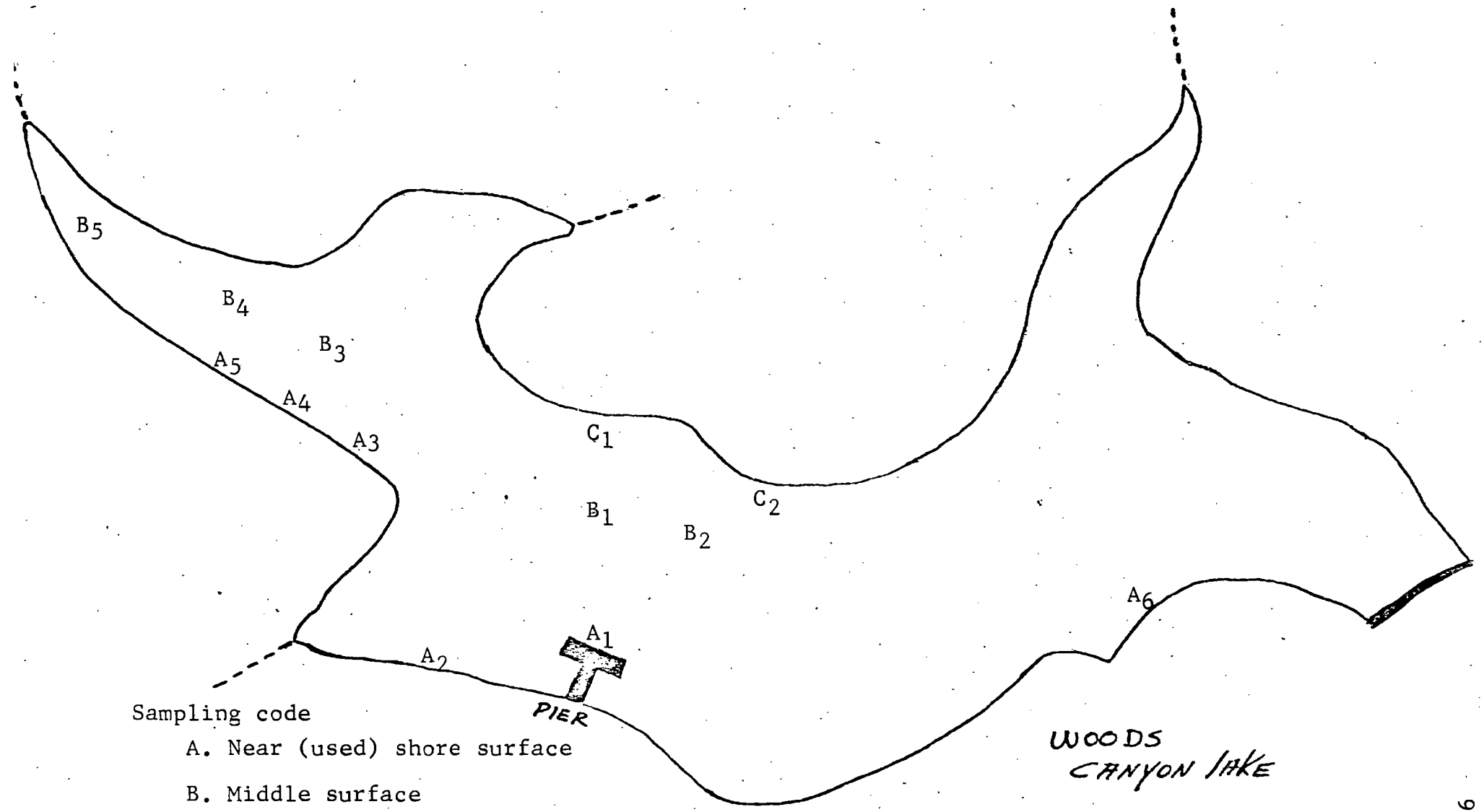
Sampling Sites:

Figures 1 and 2 are outlines of Woods Canyon Lake and Bear Canyon Lake respectively with the collecting sites indicated. The control shore site of both lakes was selected as that closest to human access from adjacent campgrounds (Bear Canyon site A_1; Woods Canyon site A_2). Samples were also routinely taken from the Woods Canyon Pier (A_1). Middle surface water samples are indicated as B sites and far shore samples as C sites.

Routine Media and Isolation Techniques:

The basic medium used for initial isolations was

Figure 1



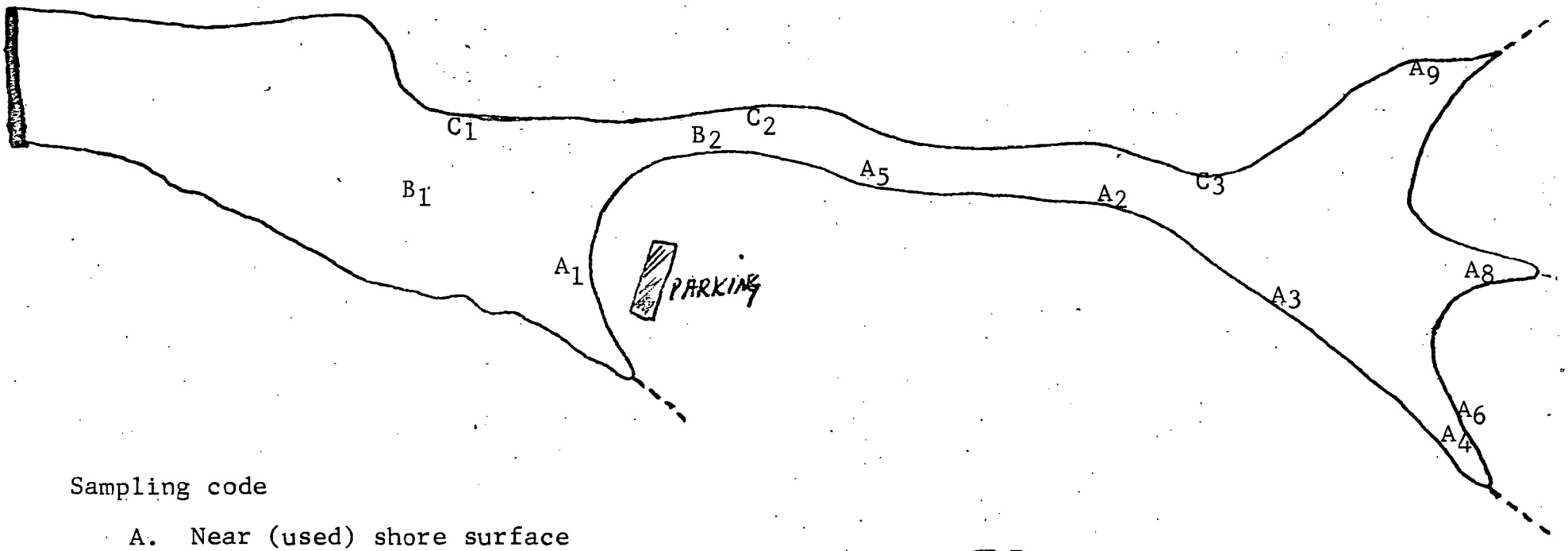
Sampling code

A. Near (used) shore surface

B. Middle surface

C. Far shore surface

Figure 2



Sampling code

A. Near (used) shore surface

B. Middle surface

C. Far shore surface

0.1% peptone (Difco) with 1.5% agar for solid medium plates. This medium was chosen to provide a selective-low nutrient medium similar to the low nutrient content of the water samples that would serve as a base line for subsequent nutritional studies. Appropriate dilutions were made in 9 ml distilled water blanks. Initial samples were plated in 0.5 ml amounts on 0.1% peptone and McConkey's (Difco) medium. This procedure avoided the heat stress effect occurring when pour plates are used (Klein and Wu, 1974). All cultures were routinely incubated at 18°C commensurate with the water temperature of the lakes. One of the initial McConkey's plates was incubated at 37°C to ascertain the effect of temperature to colony growth on this selective enteric medium. Ten ml. of all samples were inoculated into tubes containing in one case 10 ml. double strength lactose broth (Difco) and in another 10 ml. double strength azide dextrose broth (Difco). The former was incubated at 37°C overnight and observed for lactose fermentation indicative of coliform bacteria. The latter was incubated at 45°C overnight for selective isolation of fecal streptococci. Positive lactose tubes were streaked on Eosin Methylene Blue agar (EMB) (Difco) for confirmation of E. Coli. Positive azide lactose tubes were streaked on phenethyl-alcohol plates (PEA) (Difco) and incubated at 45°C for confirmation of fecal streptococci.

Following growth and colony counts on the 0.1% peptone plates these plates were replicated using velveteen pads onto 0.1% peptone plates that were subsequently incubated individually at 37°C overnight; 6°C for 3 weeks and anaerobically at 18°C for 5 days. An additional replicate was made on an EMB plate and this incubated at 37°C overnight. Counts on these plates provided comparative data for the numbers of bacteria capable of growth under these diverse conditions as compared to the control. The EMB plate provided a count of the relative number of lactose fermenting and non-lactose fermenting bacteria.

Special Media:

Several media were prepared and utilized to select for the possibility of unique groups of bacteria occurring in the lakes that would not be expected to grow under routine culture conditions.

Ammonia oxidizing bacteria (Aaronson, 1970)

A base salt medium containing $(NH_4)_2SO_4$ - 0.2 g; KH_2PO_4 - 0.2 g; $CaCO_3$ - 1.0 g; $MgSO_4$ - 0.05 g in 100 ml distilled water was prepared double strength and 10 ml sterilized in 125 ml flasks. Ten ml of sample was added to each flask which was then incubated for up to two months at 18°C.

Sulfur oxidizing bacteria (Aaronson,1970)

One gram of $Na_2S_2O_3$ was added to the above salts medium to provide an energy source for sulfur oxidizing

bacteria. These flasks were inoculated and incubated as for the ammonia oxidizing bacteria.

Photosynthetic bacteria (Aaronson, 1970)

Collected samples were added to 60 ml ground glass stoppered bottles and filled to the stopper to provide for the development of anaerobic conditions. These bottles were placed in the light at room temperature (25°C). Subsequently subcultures were made into a medium containing yeast extract 0.1 g; $Na_2S \cdot 9H_2O$ - 0.01 g; agar - 1.5 g in 100 ml distilled water. Following inoculation the tubed medium was covered with paraffin to develop anaerobic conditions and the cultures incubated at 25°C in the light.

Iron bacteria (Rodina, 1972)

To water samples containing bottom mud, crystals of ferric ammonium citrate were added and these incubated at 25°C in the dark. Subcultures were made to a medium containing NH_4NO_3 - 0.5 g; $NaNO_3$ - 0.5 g; K_2HPO_4 - 0.5 g; $MgSO_4$ - 0.5 g; $CaCl_2$ - 0.2 g and ferric ammonium citrate - 10 g in a liter of distilled water. Agar at a 1.5% concentrations was added to the above medium to provide a solid medium.

Phosphate solublizing bacteria

The phosphate medium of Rodina (1972) utilizing $Ca_3(PO_4)_2$ emulsified with gum arabic and a nutrient base of 0.1% peptone-agar was used. Bottom mud samples were

shaken and 0.5 ml samples either plated on the surface or added to pour plates of this medium. All plates were incubated at 18°C for two months and observed for clearing of the turbid medium.

Isolation and Characterization of Bacteria:

Colonies were subcultured for isolation on 0.1% peptone agar slants from plates incubated at different temperatures. Special attention was directed to isolating a representative sample of the variety of bacteria occurring on the plates as indicated by colonial morphology.

All isolates were examined for growth temperature range on 0.1% peptone media; for salt tolerance on 0.1% peptone plates containing either 3, 5 or 8% NaCl; for growth in the presence of the following selective inhibitors added to 0.1% peptone agar; sodium deoxycholate - 0.15% and potassium telurite - 0.05%. Growth on McConkeys, EMB and PEA were also determined.

The pH range of isolates was determined by inoculating tubes of 0.1% peptone broth whose pH was adjusted to 4, 5, 6, 8 and 9 and adding brom thymol blue as an indicator.

All cultures were gram stained and inoculated into glucose broth, litmus milk, nitrate medium, H_2S medium, citrate medium and Hugh-Leifson medium to establish generic characteristics. The catalase test using hydrogen peroxide and the oxidase test using Pathotec oxidase strips were also done.

Temperature of incubation for all tests was 18°C.

Chapter 3

RESULTS

Figure 3 shows the seasonal count of bacteria growing at 18°C on 0.1% peptone agar for both lakes. The count remained quite stable from June through early September at Bear Canyon while the comparable data for Woods Canyon Lake demonstrating considerable fluctuation for the same period. During this period the 78 campsites at Woods Canyon were always full as compared with 20 camp units or frequently less at Bear Canyon. Table 2 shows the collection dates and camper units estimated for each date. Transient traffic at Woods Canyon additionally provided an increased human population over that of Bear Canyon. At both lakes replicate counts made at 37°C were routinely lower than those at 18°C indicating that the indigenous bacterial flora was properly measured at this temperature which approximated lake water temperatures of 16°C to 23°C for the period measured.

Figure 4 shows the counts of colonies growing on McConkeys agar at 18°C for both lake shores. On this selective medium for enteric bacteria it is obvious that while Bear Canyon Lake remained relatively stable with only one Summer peak following the July 4 weekend, Woods Canyon showed a continuous increase with peaks in both late June

Figure 3

Bacterial counts on 0.1% peptone agar, 18°C
control shore

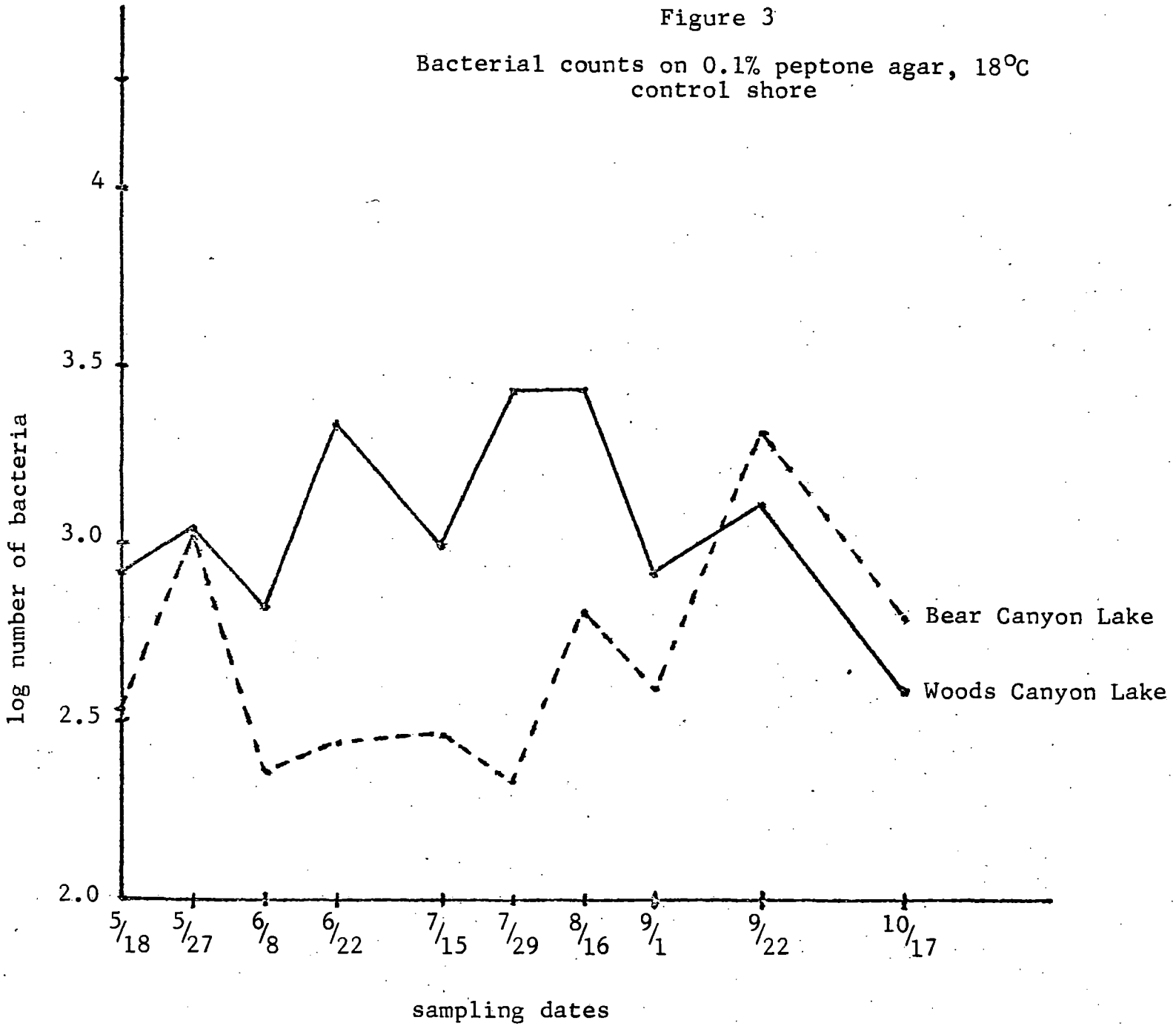


Figure 4

Bacterial counts on MacConkey's agar, 18°C
control shore

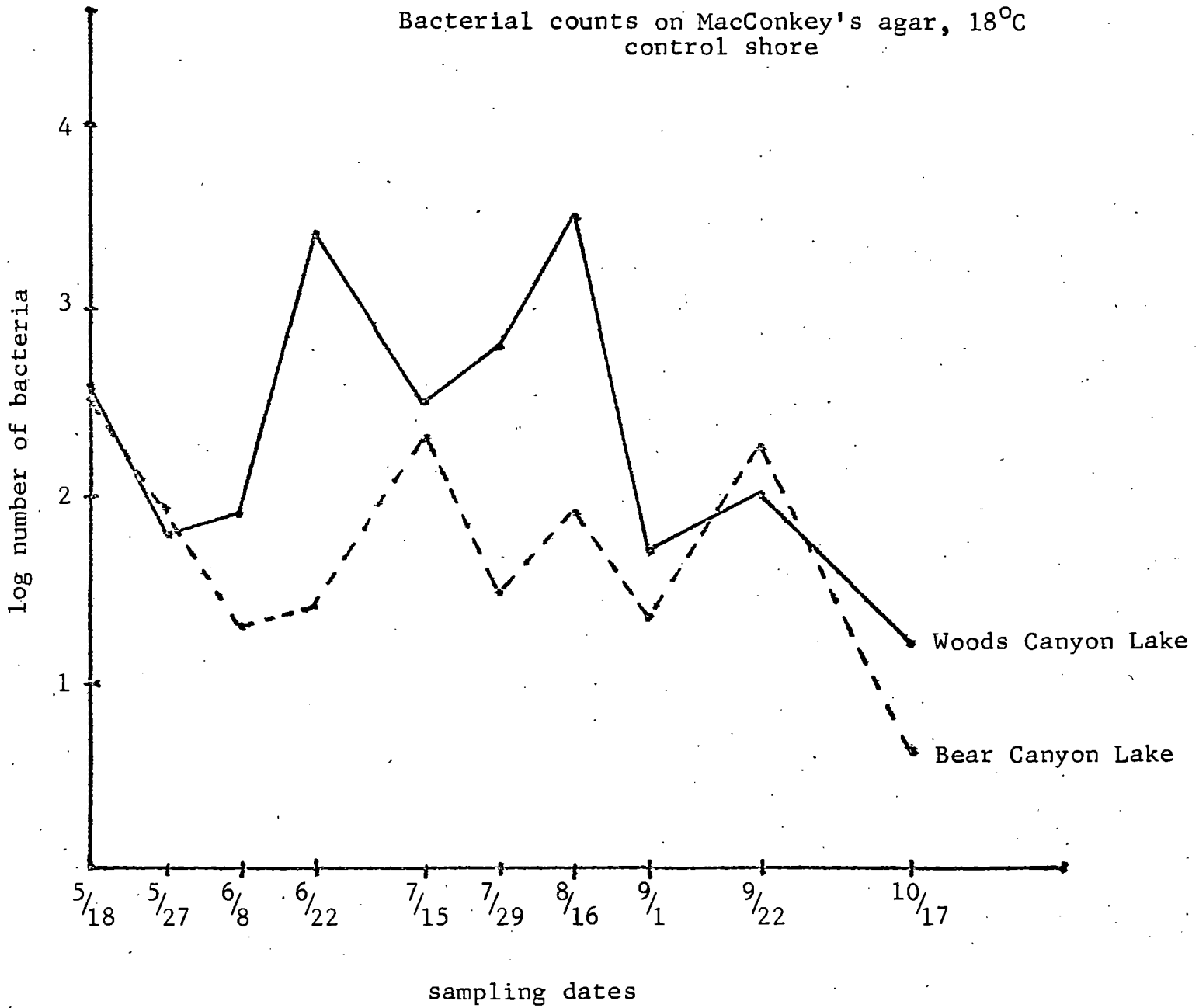


Table 2

Camper Incidence Over the Period of Study

Date	Woods Canyon Lake	Bear Canyon Lake
May 22	25 campsites used	2 units (*)
May 27	78 campsites used	50 units
June 8	"	20 units
June 22	"	20 units
July 15	"	5 units
July 29	"	2 units
August 16	"	5 units
September 1	"	20 units
September 22	"	9 units
October 17	20 campsites used	0 units

* Each tent, camper or trailer counted as one unit

and mid-August. Both lakes showed a significant decline from September to October. The two human fecal indicators Escherichia coli and Streptococcus fecalis were continuously isolated from shore samples at Woods Canyon while they occurred only from June 8 to July 29 at Bear Canyon Lake.

The above data support the basic premise of this study that Bear Canyon Lake approximated a natural forest lake receiving minimal human abuse as compared to the more heavily used Woods Canyon Lake.

Table 3 shows a comparison of bacterial counts taken from several areas of both lakes on various media and temperature conditions. Note than an average of all samples grown under the control conditions (0.1% peptone agar at 18°C) is essentially similar for both lakes. Comparison of other counts to these control counts indicate that shore samples are routinely higher in all categories except for bacteria growing at 6°C. Control shore samples at Woods Canyon Lake are routinely considerably above average except for 6°C cultures while control shore samples from Bear Canyon Lake were similar or less than the average of all samples. This indicates little shore contamination at Bear Canyon Lake as opposed to considerable shore contamination at Woods Canyon. Both center lake samples and opposite shore samples at both lakes show routinely lower counts with the significant exception of 6°C cultures from the

Table 3

Comparison of Bacterial Counts for Bear Canyon and Woods Canyon Lakes 5/18/74-8/16/74

Lake	Growth Conditions	All Samples	All Shore Samples	Control Shore Samples	Center Lake Samples	Opposite Shore samples
Bear	18°C on 0.1% Peptone	631*	706	654	314	579
Woods		728	975	1355	135	236
Bear	37°C on 0.1% Peptone	294	302	134	200	330
Woods		474	647	1296	76	83
Bear	6°C on 0.1% Peptone	309	253	148	150	597
Woods		427	181	196	107	113
Bear	18°C on MacConkey's	87	114	80	10	19
Woods		119	179	930	15	18
Bear	37°C on MacConkey's	55	84	35	3	14
Woods		86	132	1055	18	2

* Bacteria per ml.

opposite shore samples from Bear Canyon Lake. While virtually the entire shoreline of Woods Canyon Lake is used by campers the opposite shore of Bear Canyon Lake is seldom exposed to humans due to a lack of boats on the lake and the necessity of walking about one hour to reach it from the major access road.

This data support the concept that at the present time shore contamination is the major factor of pollution and the center waters remain relatively free due to both flow and dilution of shore contamination.

Table 4 illustrates the evidence of human fecal indicators at the control shores of both lakes over the period of study. Both lakes showed an increase with Summer use and a significant drop by late September and early October. The highest incidence occurred at the Woods Canyon shore on July 15 thus correlating well with the control bacterial count increase seen on 0.1% peptone agar at 18°C. Center water samples showed a low incidence of fecal bacteria on both July 29 and September 22 indicating that neither lake was promoting the growth of these species.

Table 5 shows the total count of bacteria growing on the two selective enteric media; McConkey's and EMB using two different test conditions. The McConkey's count was obtained from direct plating of the 0.5 ml. water sample while the EMB plates were replicated from colonies growing on 0.1% peptone agar. Since lactose fermenting

Table 4

Incidence of fecal indicator strains in Bear Canyon and Woods Canyon Lake shore samples

Sample Date	Lake	Number of Samples	Number of positive samples *E. coli*	*Strep. fecalis*
5/18	Bear	2	0	0
	Woods	2	1	0
5/27	Bear	2	0	0
	Woods	2	1	1
6/8	Bear	2	0	1
	Woods	2	1	1
6/22	Bear	2	1	0
	Woods	2	1	2
7/15	Bear	5	1	4
	Woods	5	4	4
7/29	Bear	2	1	0
	Woods	1	1	1
	Bear (center)	4	0	0
	Woods(center)	3	2	0
8/16	Bear	9	2	1
	Woods	3	1	3
9/1	Bear	10	0	0
	Woods	10	1	1
9/22	Bear	1	0	0
	Woods	1	1	1
	Bear (center)	3	0	1
	Woods(center)	3	0	0
10/17	Bear	3	0	0
	Woods	2	0	0

Table 5

Percentage of coliforms occurring on MacConkeys and EMB at 37°C[1]

Sampling date	Bear Canyon Total[2]	Bear Canyon MacConkeys	Bear Canyon EMB	Woods Canyon Total	Woods Canyon MacConkeys	Woods Canyon EMB
May 22	42	38%	32%	110	1%	8%
May 27	43	21%	ND[3]	30	9%	ND
June 8	10	0%	0%	46	5%	13%
June 22	5	40%	ND	76	5%	ND
July 15	57	7%	23%	91	6%	14%
July 29	17	17%	21%	307	4%	36%
August 16	28	53%	0%	164	14%	14%
September 1	7	14%	9%	9	55%	27%
September 22	42	2%	7%	18	11%	11%
October 17	4	0%	44%	1	100%	7%

[1] MacConkeys done from direct plating of sample; EMB done by replicating from 0.1% peptone agar.

[2] Total count on MacConkeys agar

[3] ND = not done

(Coliform) bacteria produce red colonies on both media a count of these provide data to calculate the percent of Coliform bacteria occurring as opposed to other gram negative bacteria. The incidence of Coliform bacteria (Table 5) is routinely low by both procedures for both lakes except in those cases where the total number is too low to provide a significant percentage figure (October 17).

The data suggest that the fecal bacteria occur in the lakes as a result of human use but do not survive well there in significant numbers.

Colonies from the 0.1% peptone agar plates incubated at 3 temperatures were picked and isolated. Appropriate examinations were done to establish their probable genus and physiological response to several ecological parameters. The results for the two lakes are shown in Tables 6 and 7. The majority are Gram negative rods characteristic of fresh water supplies (Strzelczyk et al. 1971). They tend toward being strict aerobes, with a relatively broad pH range and incapable of tolerating a 3% salt concentration in the medium. Only a few isolates capable of growth at $6^{o}C$ were also capable of growth at $37^{o}C$ and most of the $37^{o}C$ isolates were unable to grow at $6^{o}C$.

To test the temperature range of psychrophillic bacteria $6^{o}C$ cultures collected on July 29 were replicated from 0.1% peptone agar onto the same medium and incubated at $37^{o}C$. Table 8 shows that the highest percentage of

TABLE 6

Characteristics of Wood's Canyon Isolates

Temp.	Date of Isolation	Isolate	Probable Genus	Temp. Range	Salt Tolerance	pH Range	Anaerobic Growth	Glucose Fermenta
6°C		W1-4	Vibrio	6-30	0	6-9	+	+
	5/18	W1-7	Coliform	6-37	5%	4-9	+	+
		W1-10	Pseudomonas	6-30	5%	4-9	(-)	(-)
	5/27	W4-1	Flavobacterium	6-25	0	5-9	(-)	(-)
		W4-2	Pseudomonas	6-30	0	5-9	(-)	(-)
		W7-1	Flavobacterium	6-37	3%	4-9	(-)	(-)
		W7-2	Achromobacter	6-37	5%	6-8	(-)	(-)
	6/22	W7-4	Pseudomonas	6-37	3%	5-9	(-)	(-)
		W7-5	Vibrio	6-37	3%	5-8	+	+
		W7-6	Streptococcus	6-45	8%	7	+	+
18°C	5/18	W1-5	Xanthomonas	18-30	0	5-9	(-)	(-)
37°C	6/22	W7-3	Escherichia	18-37	3%	6-9	+	+
		W10-C-2	Pseudomonas	18-37	0	5-9	(-)	(-)
		W10-B2	Flavobacterium	18-37	0	7	(-)	(-)
	8/16	W10-B4	Coliform	18-37	0	7-8	+	+
		W10-A-1	Spirillum	18-37	0	5-9	(-)	(-)
		W10-A-2	Micrococcus	18-37	0	7-9	(-)	(-)

TABLE 7

Characteristics of Bear Canyon Isolates

Temp.	Date of Isolation	Isolate	Probable Genus	Temp. Range	Salt Tolerance	pH Range	Anaerobic Growth	Glucose Fermentation
6°C		B2-2	Flavobacterium	6-25	0	5-7	(-)	(-)
		B2-4	Pseudomonas	6-37	5%	4-9	(-)	(-)
	5/18	B2-5	Pseudomonas	6-30	0	4-9	(-)	(-)
		B2-7	Pseudomonas	6-30	0	5-9	(-)	(-)
		B2-8	Achromobacter	6-30	5%	4-9	(+)	(+)
		B4-1	Pseudomonas	6-37	0	4-9	(-)	(-)
	5/27	B4-2	Flavobacterium	6-37	0	4-9	+	+
		B4-3	Flavobacterium	6-30	5%	4-9	(-)	+
		B3-1	Pseudomonas	6-30	0	4-9	(-)	(-)
		B7-A-1	Pseudomonas	6-30	0	5-9	(-)	(-)
	6/22	B7-D-1	Achromobacter	6-30	3%	6-9	(+)	(-)
		B7-D-3	Micrococcus	6-37	3%	5-9	(-)	(+)
		B10-A1	Coliform	6-25	0%	5-9	(+)	(+)
	8/16	B10-F1	Pseudomonas	6-25	0	7-9	(-)	(-)
18°C	6/22	B7-C1	Pseudomonas	18-25	0	6-8	(-)	(-)
	8/16	B10-I-1	Pseudomonas	18-25	0	5-9	(-)	(-)
37°C		B7-C-2	Bacillus	18-37	0	6-7	+	+
		B7-D-2	Achromobacter	18-37	0	5-9	(-)	(-)
		B7-6	Bacillus	18-37	0	6-8	(-)	(-)
	6/22	B7-7	Pseudomonas	18-37	0	5-8	(-)	(-)
		B7-8	Bacillus	18-37	0	6-8	(-)	(-)
		B7-1	Achromobacter	18-37	0	6-8	(-)	(-)
		B7-2	Pseudomonas	18-37	0	6-8	(-)	(-)
		B7-3	Flavobacterium	18-37	0	5-9	(-)	(-)
		B7-5	Pseudomonas	18-37	0	4-9	(-)	(-)
	8/16	B10-D-1	Coliform	18-37	0	4-9	(+)	(+)

Table 8

Replication of 6°C cultures for growth at 37°C on 0.1% peptone medium*

Source	Percent growing at 37°C
Woods control shore	82
Bear control shore	64
Woods center water Two samples	77
Bear center water Two samples	53
Bear far shore	54

* Samples collected July 29

bacteria capable of growth at both temperatures occurred in Woods Canyon Lake while in the center water samples from Bear Canyon Lake only about one-half of the bacteria had this temperature range. Shore samples at both lakes were higher for bacteria growing over this temperature range than the corresponding center open water samples. Compared with the previous data on isolated cultures this indicates a relatively high summer incidence of those few species capable of growth over the tested temperature range and their increase occurrence in shallow shore water.

This aspect of the study was done with a view to developing a selective medium and/or conditions for estimating the status of normal indigenous bacteria in the water. The data demonstrates that 0.1% peptone medium containing 3% salt incubated at 37°C under anaerobic conditions would effectively inhibit growth of most indigenous bacteria thus the demonstration of bacteria under those conditions would be indicative of polluting forms and could be used as a rapid screening method.

Two bacterial species readily identifiable as a result of their pigmentation were found in all samples plated for counts except one (Bear Canyon Lake - Sept. 1). Chromobacter lividum produces a dark purple colony on 0.1% peptone when incubated at 18°C for 3-5 days and Cytophaga johnsoni produces yellow swarming colonies under the same conditions. Either or both of these bacteria can

be used as indicative of good quality water and their absence would be suggestive of pollution. The characteristics of these two species are listed in Table 9.

By replicating the plates used for counts onto 0.1% peptone agar and incubating at 6°C it was possible to establish the percent of total bacteria capable of surviving low temperatures. Table 10 shows that a significant proportion of these bacteria are present during the Summer months providing a stable year around flora for the lake.

Similarly replicate plates incubated anaerobically from July through October show a marked decrease in the ability of the isolated strains to grow anaerobically as use of the lakes decreases (Table 11). Increasing anaerobic conditions is a common result of organic pollution and would encourage the growth of faculatative or anaerobic bacteria. The fact that this does not occur as the season wanes indicates that both lakes are presently capable of recovering from Summer organic pollution factors.

To establish broader bacteriological parameters for the lakes attempts were made using selective media to isolate nitrifying bacteria, sulfur utilizing bacteria, iron bacteria and photosynthetic bacteria. Mud-water shore samples collected in May and July were used as a source and all were negative for the above groups of bacteria. Mud and water samples collected from May through mid-July

Table 9

Characteristics of potential indicator bacteria

Characteristic	*Chromobacterium lividum*	*Cytophaga johnsoni*
Morphology	Gram (-) Rod	Gram (-) Rod
Pigment (0.1% peptone)	Deep blue	Yellow spreading
Glucose	Acid	Acid
Nutrient agar	Growth: no pigment	Growth-not spreading
Trypticase Soy agar	No growth	Yellow-not spreading
Anaerobic growth	(-)	(-)
pH range	5-9	5-9
Growth 37°C	(-)	(-)
Growth 6°C	(+)	(+)
Nitrate reduction	Reduced	(-)
3% NaCl	No growth	No growth
MacConkey's agar	No growth	No growth
Eosin-Methylene blue agar	No growth	Growth
Oxidase test	(-)	(-)
Citrate test	(-)	(+)

Table 10

Percent of total isolates growing at 6°C

Date	Bear Canyon Lake control shore	Woods Canyon Lake control shore	Woods Canyon Lake pier
6/8	53	54	62
6/22	100	100	100
7/15	27	30	42
7/29	59	66	ND*
8/16	25	50	42
9/1	34	18	22
9/22	50	22	18
10/17	32	55	28

* Sample froze - uncountable

Table 11

Percent of total isolates

showing anaerobic (faculatative) growth

Date	Bear Canyon Lake		Woods Canyon Lake	
	samples	percent	samples	percent
7/15	5	45	5	54
7/29	6	18	4	40
8/16	9	12	3	19
9/1	2	27	2	25
9/22	4	11	4	12
10/17	3	7	2	13

were examined for phosphate solubilizing activity and all were negative. This lack of diverse bacteria combined with the relatively low counts of indigenous bacteria would indicate that both lakes have a low nutrient content indicative of good quality water.

As a major purpose of this work was to establish the characteristics of the indigenous bacterial flora several bacterial species were isolated and inoculated into filtered-sterile Bear Canyon Lake water and incubated at 18°C. As shown in Table 12 several of these isolates, including Chromobacterium lividum and Cytophaga johnsoni were propagated through four subcultures over a three month period. Significantly neither Streptococcus fecalis nor Escherichia coli isolates survived under these conditions.

As a correllary experiment four strains were inoculated into 100 ml filtered-sterile Bear Canyon Lake water cultures and plated to establish their growth potential at 18°C. The results in Figure 5 show that three of the 4 including the two indicator strains were capable of growth under these conditions.

These later experiments establish the ability of the indigenous bacterial flora to survive and multiply under conditions extant in the lakes while human fecal bacteria cannot.

Table 12

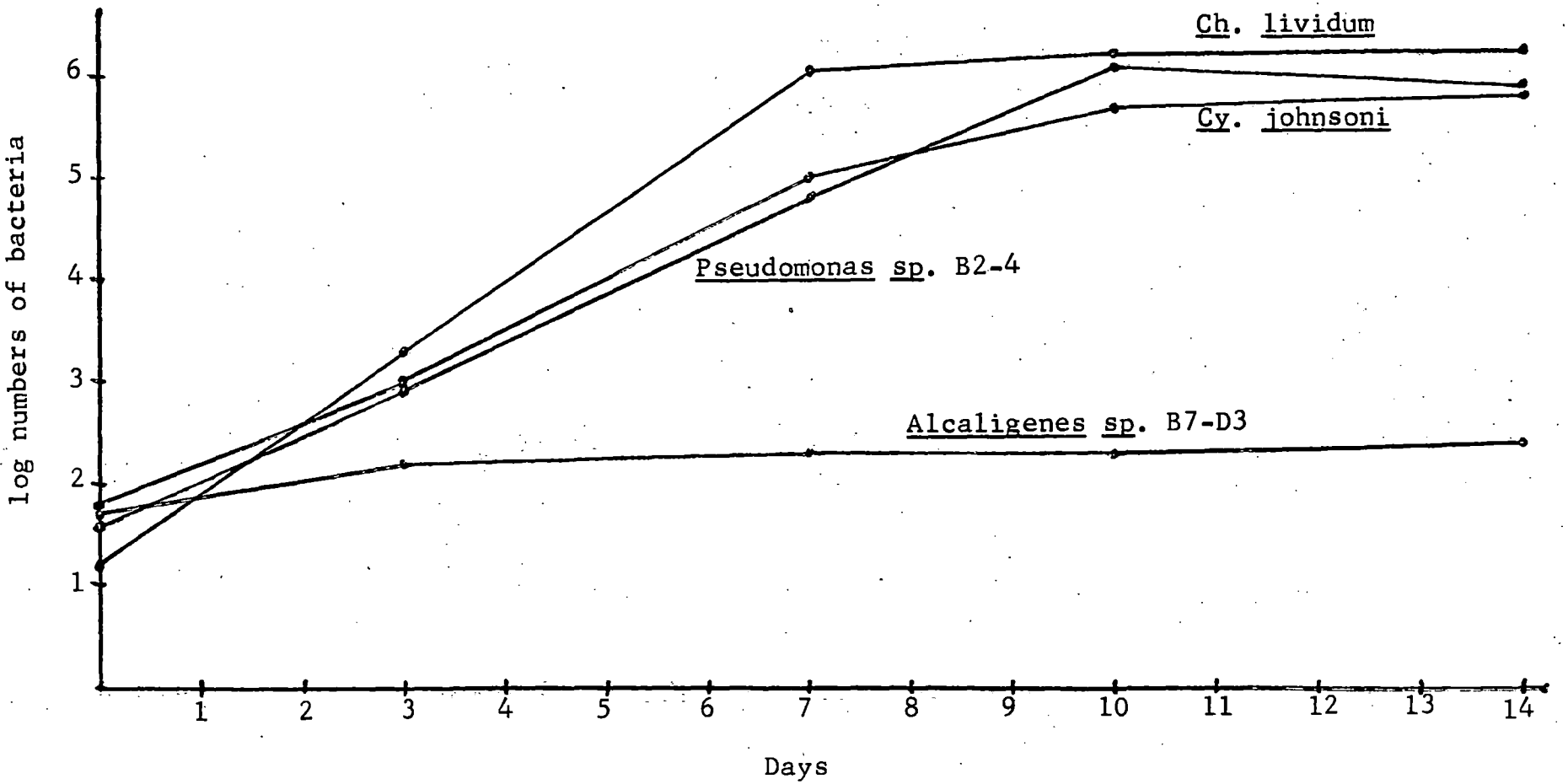
Survival of bacterial isolates in lake water

Bacterial species	Dates of subculture				
	10/25	11/3	12/4	1/13	1/24
Chromobacterium lividum	+*	+	+	+	+
Cytophagh johnsoni	+	+	+	+	+
Pseudomonas sp.	+	+	+	+	+
Pseudomonas sp. W1-10	+	+	+	+	+
Pseudomonas sp. B2-4	+	+	+	+	+
Xanthomonas sp. W1-5	+	+	+	+	+
Alcaligenes sp. B7-D3	+	+	+	+	+
Alcaligenes sp. B7-1	+	+	+	(-)	(-)
Escherichia coli W7-3	+	(-)	(-)	(-)	(-)
Streptococcus fecalis W7-6	+	(-)	(-)	(-)	(-)
Bacillus sp. B7-8	(-)	(-)	(-)	(-)	(-)
Flavobacterium sp. B7-4	(-)	(-)	(-)	(-)	(-)

* Growth on 0.1% peptone agar when subcultured from lake water tube.

Figure 5

Growth of bacterial isolates in lake water, 18°C



Chapter 4

DISCUSSION

Utilizing all parameters measured Woods Canyon Lake showed a higher indication of human abuse than Bear Canyon Lake. These included a higher total incidence of all bacteria as well as coliform bacteria. There was also a greater fluctuation in counts at Woods Canyon Lake particularly during peak Summer months indicating variable introduction of both total and coliform bacteria due to human use. During the Summer period bacteria capable of both growth at 37°C and under anaerobic conditions reach higher numbers at Woods Canyon Lake as compared to Bear Canyon Lake indicative of the greater human use at the former lake.

Conversely both lakes maintain a significant population of psychrophillic bacteria throughout the Summer season and the incidence of strictly aerobic bacteria increases as the season wanes indicating a good recovery following human use. By comparison Larkin (1970) found the psychrophillic population of a Louisiana lake virtually disappeared during the Summer months. Another study (Gordon, 1972) in a subarctic Alaskan river indicated that although both E. coli and enterococci decreased in

numbers, two to eighteen percent of these bacteria remained viable after seven days at the 0°C conditions in the river.

Comparing bacterial counts of shore water to open water counts are routinely higher for both lakes in the shore samples throughout the season indicating that the open water is not subject to significant human abuse from either boat use or swimming. In addition the source of shore pollution is apparently not capable or high enough to effect center lake waters. Woods Canyon Lake shore sample counts are always higher than Bear Canyon Lake shore samples which correlates well with the increased use of the Woods Canyon Lake shore.

Fecal indicator bacteria occur erratically in both lakes and show no tendency to establishing themselves but conversely decline significantly in open waters and at the end of the season in both lakes. Nutrients present in the lake water as shown in this study are not capable of supporting growth of either of the two fecal indicator strains.

Bacterial genera isolated from both lakes are characteristic of clean recreational waters and can be categorized as saprophytic Gram negative bacilli. As a group their physiological characteristics are essentially strict aerobes, growing over a pH range of 5 to 9, incapable of tolerating 3 percent salt and preferring a maximum temperature of 25°C. This data provides a basis for

developing a selective medium and physiological conditions that would inhibit the growth of these normal bacteria such that bacterial growth on this medium would be directly proportional to influx of abnormal bacteria and indicative of polluting species. While there is a need to field test this approach over a larger group of recreational lakes indications are that it could provide an "early warning system" of water quality deterioration prior to the advent of significant numbers of fecal indicator bacteria. While the significance of fecal indicator bacteria in drinking water supplies is unquestioned their presence in recreational waters is almost to be anticipated and their significance therefore dependent more on quantity than simply occurrence (Bott, 1973).

Two species of bacteria found routinely in both lakes are also suggested as indicator strains for good quality recreational water. Both have the indicator advantage of being pigmented and hence readily identifiable in a routine sampling procedure. Here again more extensive field testing of the incidence of these species in a variety of recreational waters would be instructive however based on their known physiological characteristics they could be expected to disappear from recreational waters at an early stage of pollution.

The apparent lack of bacteria capable of producing sulfates, nitrates or utilizing iron correlated well with

the concurrent chemical analysis of both lakes water reported by Segall and Oakley (1975). They found these compounds essentially absent or below detectable amounts in both lakes. Phosphates were also reported as undetected in Bear Canyon Lake and the surface waters of Woods Canyon Lake and this correlates with the lack of phosphate solubilizing bacteria reported.

While both lakes are subjected to varying degrees of human use at the present all bacterial parameters studied indicate that both lakes are presently able to recover from the current human use and that some increased use of Bear Canyon Lake would not be harmful at this time except from an esthetic standpoint.

Ironically studies have shown (Warren et al., 1964) that some increase in organic and/or pollutant factors may be responsible for increased fish productivity in that such organic material provides for increased bacterial growth of species used as feed for larvae that are subsequently food for the fish population. The best studied bacteria in this regard Sphaerotilus natans (Stokes, 1954) has often been considered an undesirable pollutant bacteria and it was not found in either lake in this study. This study combined with the chemical data reported by Segall and Oakley (1975) point to the dilemma of providing healthful recreational waters with high fish populations in artificially constructed lakes.

While algae and photosynthetic potential are commonly used as indicators of primary productivity of natural waters (Fjerdingstad, 1971) the relationship of the food chain commencing with organic pollutants to non-photosynthetic, chemosynthetic bacteria as a food source for invertebrates that supply the nutritional needs of game fish has had little study. Controlled increase of organic material to these lakes may well be possible with a minimal health hazard effect and yet provide an increase in fish productivity. That selected species of bacteria do in fact provide a good source for some invertebrate larvae has been established in marine shore environments (Gray and Johnson, 1970). Further studies in fresh water lakes toward this end would appear to be a worthwhile endeavor of the U. S. Forest Service in providing for both recreational waters and fish productivity.

Chapter 5

SUMMARY AND CONCLUSIONS

Two, man-made, forest lakes located in a ponderosa pine forest on the Mogollon rim in Arizona were examined bacteriologically during the Summer of 1974. Total bacterial counts were made from May through October on a medium and with conditions similar to those extant in the lakes. Coliform bacteria and fecal streptococci were also enumerated along with attempts to isolate specialized bacteria associated with geochemical cycles. Isolates were characterized biochemically to provide characteristic parameters for growth of the indigenous lake bacteria and from this data conditions proposed for a screening method to evaluate the influx of bacteria from increased human abuse. Two bacterial species were characterized and recommended for use as indicator bacteria of good quality water.

The data obtained support the following conclusions:

1. That conditions used for enumerating indigenous water bacteria were optimal.
2. That Woods Canyon Lake, receiving the heaviest human use compared to Bear Canyon Lake, had a more fluctuating number of bacteria and a higher and more prolonged incidence of fecal indicator bacteria.

3. That at both lakes shore contamination is the major factor of bacterial pollution and that center waters remain relatively free due to both flow and dilution of shore contamination.

4. That fecal indicator bacteria occur in the lakes as a result of human use but do not survive there in significant numbers.

5. That a bacterial population capable of growth at 6°C survives the entire Summer in numbers sufficient to reestablish a winter flora.

6. That selective conditions can be established using a 0.1% peptone medium that would indicate an increase in pollutant bacteria prior to the time that fecal indicator bacteria occur in significant numbers.

7. That readily identifiable pigmented bacteria are available as indicators of good quality water and that these strains are capable of growth using the lake water as the sole medium.

8. That although all indicators studied indicate the increased use effect on Woods Canyon Lake over Bear Canyon Lake, both lakes recover from this use by mid-October.

9. The apparent absence of bacterial types associated with geochemical cycling correlates well with the low concentrations or absence of the compounds involved as determined by the correllary Segal-Oakley

study. Both studies emphasizing the inherent present low nutrient quality of both lakes.

10. That, asthetic considerations aside, the use of Bear Canyon Lake can be significantly increased to a level reaching Woods Canyon Lake and yet be expected to recover to a normal state on an annual basis.

11. That experiments or treatments could be designed that would increase the fish productivity of these lakes with a minimal harmful effect on lake deterioration or esthetic value. An increased understanding of the food chain relationship between bacteria, larvae and trout would be productive in this regard.

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EISENHOWER CONSORTIUM

VIII B-2

Abstract of Final Report

Cooperative Agreement 16-444-CA
Arizona State University
Roy M. Johnson, Principal Investigator

Johnson, Roy M.
1975. Development of a practicable system for monitoring the microbiological characteristics of forest lakes. 45 p. (mimeo.)

Bacterial counts were made in two man-made forest lakes in Arizona; one receiving heavy recreational use and one receiving only light use. It was found that the lake receiving the heaviest use had the highest levels of fecal indicator bacteria and the highest levels of contamination occurred along the shores of both lakes. Also, while fecal indicator bacteria occur in lakes as a result of human use, they do not appear to survive there in significant numbers.

The research revealed that the presence of Chromobacter lividum and Cytophaga johnsoni can be used as indicative of good quality and their absence as suggestive of pollution. Testing for these two species of bacteria could be used as a rapid screening method of determining water quality.